



SHORT REPORT

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The L76V mutation in HIV-1 protease is potentially associated with hypersusceptibility to protease inhibitors Atazanavir and Saquinavir: is there a clinical advantage?

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Abstract

Background: Although being considered as a rarely observed HIV-1 protease mutation in clinical isolates, the L76V-prevalence increased 1998-2008 in some European countries most likely due to the approval of Lopinavir, Amprenavir and Darunavir which can select L76V. Beside an enhancement of resistance, L76V is also discussed to confer hypersusceptibility to the drugs Atazanavir and Saquinavir which might enable new treatment strategies by trying to take advantage of particular mutations.

Results: Based on a cohort of 47 L76V-positive patients, we examined if there might exist a clinical advantage for L76V-positive patients concerning long-term success of PI-containing regimens in patients with limited therapy options. Genotypic- and phenotypic HIV-resistance tests from 47 mostly multi-resistant, L76V-positive patients throughout Germany were accomplished retrospectively 1999-2009. Five genotype-based drug-susceptibility predictions received from online interpretation-tools for Atazanavir, Saquinavir, Amprenavir and Lopinavir, were compared to phenotype-based predictions that were determined by using a recombinant virus assay along with a Virtual Phenotype™(Virco). The clinical outcome of the L76V-adapted follow-up therapy was determined by monitoring viral load for 96 weeks.

Conclusions: In this analysis, the mostly used interpretation systems overestimated the L76V-mutation concerning Atazanavir- and SQV resistance. In fact, a clear benefit in drug susceptibility for these drugs was observed in phenotype analysis after establishment of L76V. More importantly, long-term therapy success was significantly higher in patients receiving Atazanavir and/or Saquinavir plus one L76V-selecting drug compared to patients without L76V-selecting agents ($p = 0.002$).

In case of L76V-occurrence ATV and/or SQV may represent encouraging options for patients in deep salvage situations.

Background

The reduced susceptibility to certain antiretrovirals is often accompanied with a gradual loss of viral fitness, indicating that mutations with high fitness costs are less able to persist in the absence of drug pressure [1]. There have been recent reports about HIV strains with increased susceptibility to particular drugs when certain

mutation patterns had developed under antiretroviral treatment [2-5]. This biological attribute enables new putative strategies for future treatment of HIV-infected patients with abundant resistance mutations by trying to take advantage of particular mutations [6].

As example, M184V/I, the most prevalent NRTI-mutations selected under 3TC or FTC in the reverse transcriptase, do for instance revert partially the effect of thymidine-analogue mutation- (TAM) on resistance [7]. K65R and L74V are further mutations which can confer hypersusceptibility or resensitization to AZT [8].

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Beside these specific mutations in the reverse transcriptase, there are also reports about resensitizing mutations affecting the protease gene [9,10].

Objectives

This article reports about possible clinical advantages of a valine substitution, instead of leucine, at position 76 in the HIV-1 protease. This mutation generally disappears quickly in replicating viruses in absence of selection pressure mediated by LPV, APV or DRV treatment. Thus, for deep salvage therapy situations in patients with strongly limited therapy options, it might be of advantage to maintain these drugs in treatment regimens to preserve L76V in the current replicating virus in combination with a “resensitized” drug ATV or SQV.

Results

Patients with protease gene mutation L76V show increased susceptibility for Atazanavir and Saquinavir
At first, the impact of L76V on ATV- and SQV-resistance characteristics was assessed before and after

establishment of the mutation. Due to the manifestation of the L76V mutation as well as other minor mutations at resistance-relevant sites in the course of treatment, genotype-based interpretation tools predicted intermediate or mostly complete resistance against all PIs including ATV and SQV and the majority of NRTIs and NNRTIs resulting in an active drug score (ADS) of ≤ 1.0 for the failing regimen (Figure 1). Interestingly, in phenotypic analysis, the resistance factor (RF) for ATV and SQV remained at full susceptibility in both patients and even decreased for SQV from 31 to 1.1 (Figure 1A) and 1.1 to 0.6 (Figure 1B) and for ATV from 62 to 2.8 and 4.3 to 0.9, respectively.

In a further aspect, genotypic and phenotypic resistance data of 10 patients, all L76V positive, was assessed in order to analyze if these observed resensitizing effects represent ubiquitous drug resistance patterns. Figure 2 supports this hypothesis on a variety of other patients harbouring HIV populations with L76V mutation. The accuracy and concordance of predicted genotype-based interpretations were compared with obtained phenotypic

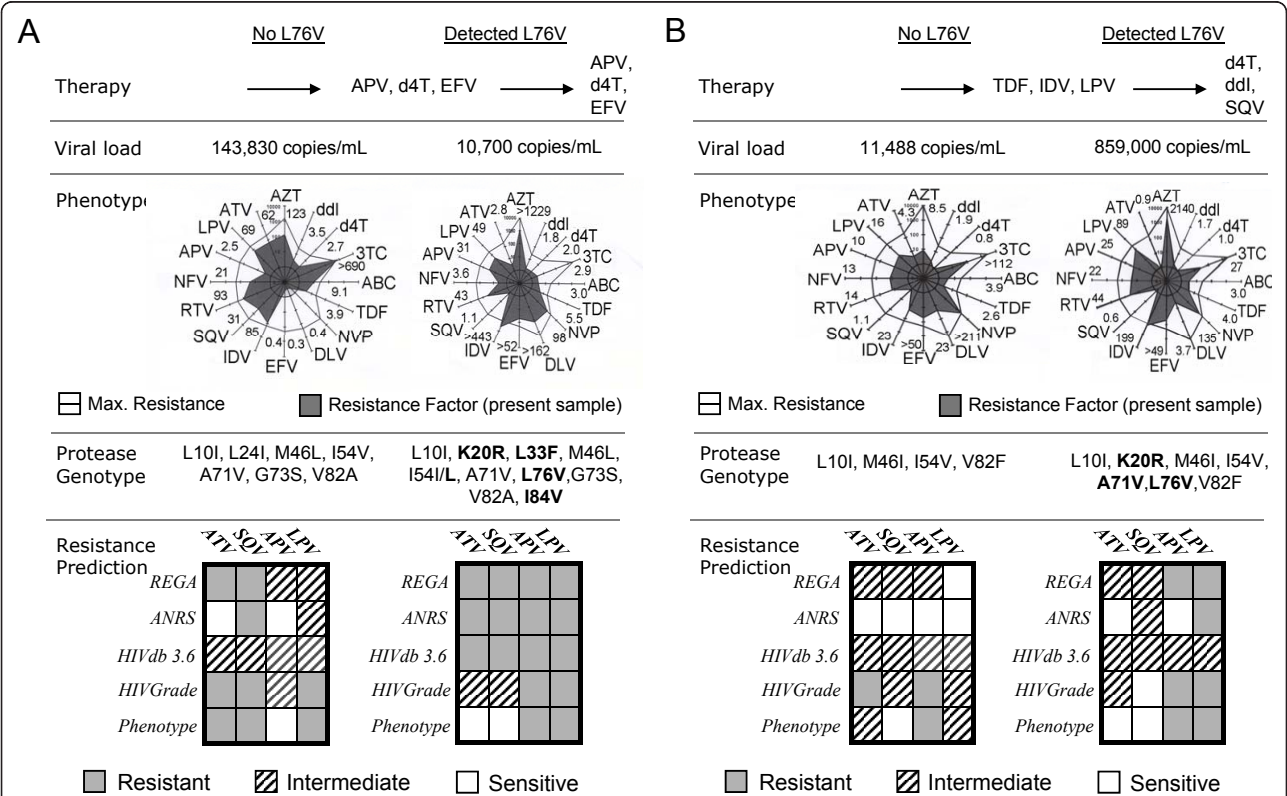


Figure 1 Resensitizing effects of the L76V mutation are visible in phenotype results: Phenotypic resistance analysis before and after manifestation of L76V in two representative patients (A+B). Although additional mutations developed in the progress of therapy (bold characters) the resistance factor for ATV and SQV decreased below the cut-off for full susceptibility in both patients compared to analyses one year before. Antiretroviral drugs are illustrated with corresponding resistance factors (cut-off: 0-3.5 = sensitive 3.6-9.5 (29 for LPV) = intermediate; >9.0 (29 for LPV) = resistant). Genotypic resistance interpretations derived from five common online tools showed considerable discrepancies in weighting of ATV and SQV resistance levels compared to each other and to phenotypic results (grey and white colour). **A)** One patient with failing APV containing therapy after week 72. **B)** Another patient with a failing IDV/LPV treatment before start of SQV containing therapy.

resistance levels from recombinant virus assays and virtual phenotype analysis.

Despite a general concordance in genotype- and phenotype-based resistance predictions for LPV and APV, there were wide discrepancies in the weighting of resistance for ATV and SQV, mostly overestimation of resistance in genotype-based predictions (Figure 2). However, most phenotypic resistance interpretations uncovered full susceptibility for the drugs ATV and SQV. In most cases L76V appeared to be associated with a variety of other resistance relevant protease mutations without effecting the resensitizing effect. However, in particular, the copresence of the protease mutation L90 M was notably associated with high ATV and SQV resistance factors (Figure 2; #4, #26, #21).

Clinical outcome and follow-up in patients with L76V-adapted therapy

Considering the effect of L76V on susceptibility for ATV and SQV, the big question was obviously, how this mutation might affect the therapeutic option and strategy for patients with a narrow margin of remaining active drugs. A considerable issue remained to generalize data from a small cohort of patients with diverse optimized backbone therapies. Thus, this work focused on the amount of active drugs in the treatment of each patient. Table 1 shows the L76V-adjusted follow-up therapies that were administered after resistance prediction results.

Sufficient virus suppression below the detection limit was initially observed in 50% of group A (ATV and/or

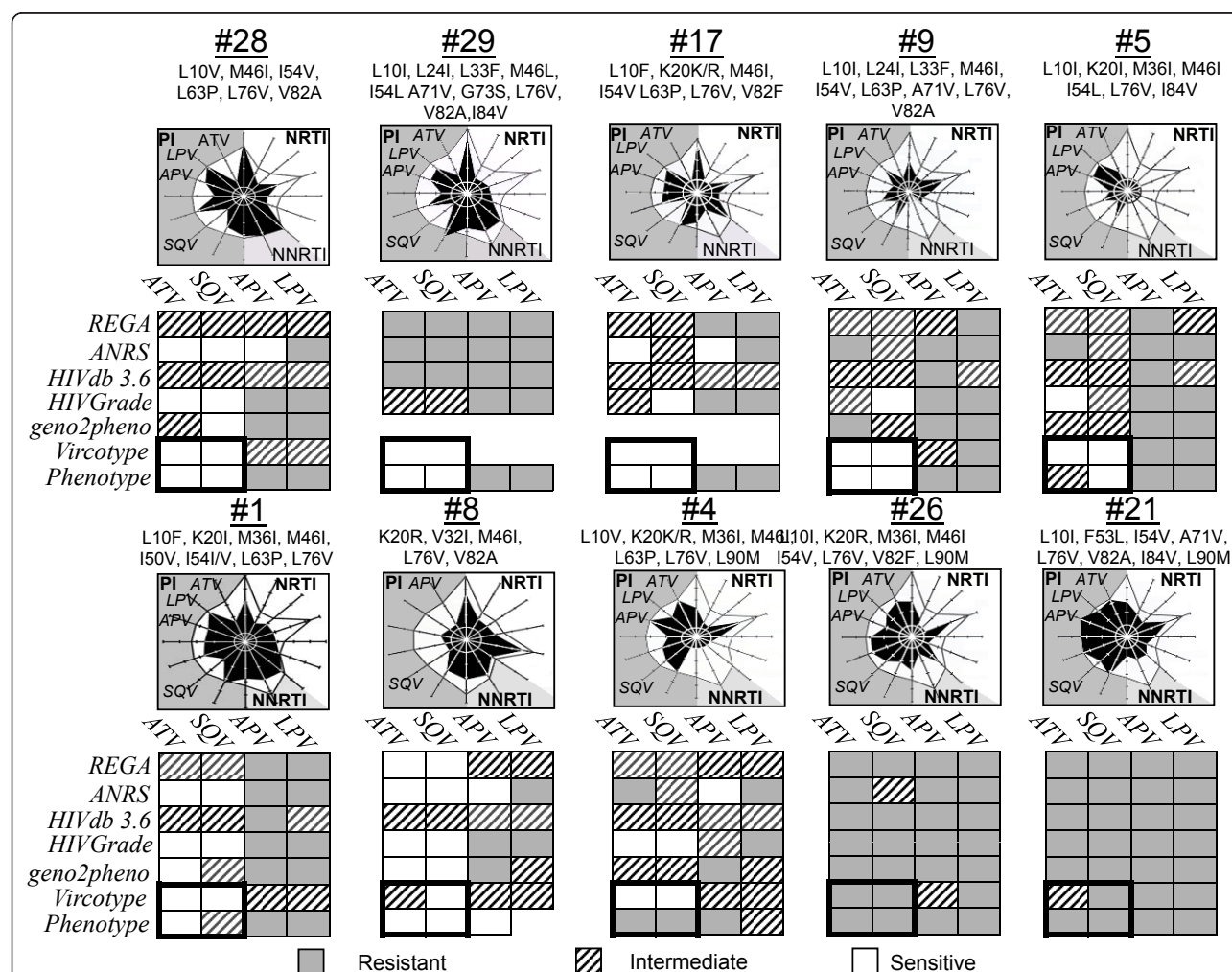


Figure 2 The "resensitizing" effect of 76V could be observed in a variety of other patients before start of PI-containing therapy. Genotypic and phenotypic data of 10 representative PI-experienced patients were analysed by using five common resistance interpretation systems Stanford HIVdb 4.3.6; REGA v7.1.1, HIV-Grade 04/2008, ANRS 10/2007 and geno2pheno. Genotypic resistance results were compared to phenotypic resistance results derived from recombinant virus assay results and/or Virtual Phenotype™ analysis (Virco).

SQV without L76V-selecting drug) and 67% of group B (ATV and/or SQV plus L76V-selecting drug LPV or APV) within the first weeks of follow-up therapy. Despite similar response rates at first, a sustained therapy success with virus suppression still below 50 copies/mL at week 96 and longer was predominantly achieved in group B patients where the selection pressure on L76V was constantly maintained by the drugs LPV or APV (Table 1, lower rows). While 66.7% of group B patients remained under detection levels at week 96, there was a significantly lower success rate in group A patients with 16.7% remaining <50 copies/mL in per-protocol analysis ($p = 0.002$; χ^2 test) (Table 2 and Figure 3). Patients of group C did not show any virus suppression below detection limit.

Interestingly, despite a successful virus suppression <50 copies/mL, the majority of group B therapies were expected to have an ADS below 2.0 after genotypic resistance predictions, indicating a very likely event of therapy failure (bold numbers in square brackets) (Table 1).

Most of the cases where therapies were predicted to have active drug scores of ≥ 2.0 turned out to be successful. Only few experienced virological failure despite an ADS of more than 2.0 (indicated in round brackets).

A major question remained obviously, why patients of group A display earlier therapy failures than patients of group B. After two years of follow-up therapy, only one patient of group A, who additionally received a fusion-inhibitor containing treatment, showed a viral load still below 50 copies/mL. The median viral load increased after 24 weeks of follow-up in group A (Figure 3). More interestingly, due to a loss of selection pressure on the L76V mutation, it was then undetectable in those patients who failed therapy, resulting in a decrease of the ADS below <2.0 (Table 3). While L76V was undetectable in patients where no L76V-selecting drug was applied, it persisted in group B and group C where selection pressure on mutation L76V was maintained (Table 3). In these patients the therapy failure had other reasons (e.g. acquisition of L90M).

These results additionally indicate benefits for patients with L76V-selecting drugs in combination with L76V-"resensitized" drugs. A major issue remains the establishment of additional protease gene mutations i.e. L90 M and further compensatory changes over the time (Figure 2; #4, #21, #26), making it crucial to suppress the virus completely and monitor viral load in close intervals.

Discussion

Little is known about the impact of drug-resensitizing mutations on antiretroviral therapy. Most works mainly describe the effects of resistance mutations on reductions in drug susceptibility. However, selective pressure

of drug therapy may also lead to shifts in the quasispecies distribution and fitness of those mutants with decreased sensitivity to the respective antiretrovirals [11,12]. This loss in replication fitness may be even larger for a multi-drug resistant virus and might lead to a better starting point for particular antiretroviral regimens [13]. Nevertheless, it is not always applicable that the acquisition of drug-resistance mutations inevitably result in loss of viral fitness. Even in case a loss is apparent, the virus may select compensatory changes over time [12,14]. This may explain why current treatment guidelines still advocate a switch to antiretroviral treatment regimens following the emergence of drug resistance mutations and possibly prior to selection of compensatory changes [11]. In summary, drug hypersusceptibility mutations which reduce viral fitness are difficult to maintain in the predominant virus population in multiple pretreated patient.

In this article we provide insights into a possibility how to maintain efficient selection pressure on the protease mutation at position L76V by combining one drug, which selects L76V (in this case LPV, APV, DRV) and another drug which gains efficiency when L76V develops. This article reports about a significant clinical benefit of the protease mutation L76V on drug susceptibility to ATV and SQV due to resensitizing effects in multi-resistant patients resulting in a significantly higher long-term therapy success. These results may be in line with explanations from molecular dynamics- and free energy studies recently reported by Alcaro et al. who found that in the presence of the L76V substitution, ATV reveals a more productive binding affinity, in agreement with hypersusceptibility data [15].

Conclusion

The strategy of combining mutation-selecting drugs with "resensitized" drugs has already been discussed for the reverse transcriptase mutation M184V in NRTI-containing therapies [6,13,16] and has also been shown to be an adequate option for a couple of other mutations including the mutation N88 S [10].

Despite initially adequate therapy response rates in 50% (group A) - 67.7% (group B) of cases, it remains a major issue that virological failure under these therapies often occur due to compensatory changes in the virus genotype over the time mostly due to an additional establishment of further mutations in the respective gene [12,14]. As shown in Table 3, failure of therapy in L76V-positive patients with ATV and/or SQV containing therapy was noticeable associated with an additional establishment of the protease gene mutation at position L90 M which resulted in resistance against all available PIs [17]. Six of eight patients who received a second genotypic resistance test following

Table 1 Active drug score (ADS) for the follow-up therapy

GROUP A																	GROUP B											
Pat-ID	#1	#2	#4	#12	#14	#17	#18	#21	#22	#46	#3	#5	#8	#9	#10	#11	#16	#19	#24	#25	#26	#27	#31	#33	#37	#39	#40	#41
Therapy	ATV	ATV	ATV	ATV/r	SQV	ddl	ATV	ATV	ATV/r	SQV/r	LPV/r	LPV/r	LPV/r	LPV/r	LPV	LPV/r	LPV/r	LPV/r	APV	APV	APV/r	LPV/r	LPV/r	LPV/r	LPV/r	LPV/r	LPV/r	LPV/r
	SQV	SQV	SQV	SQV	TPV/r	d4T	SQV	EFV	SQV	r	SQV	ATV	SQV	SQV	ATV	ATV	SQV	SQV	SQV	SQV	SQV	SQV	SQV	SQV	SQV	SQV	ATV	
	RTV	RTV	RTV	3TC	3TC	SQV	3TC	TDF	AZT	TDF	3TC	AZT	d4T	ENF	FTC	EFV			TDF	ddC		FTC	3TC	AZT	ddl	TDF	TDF	TDF
	FTC	3TC		T20	ddl		T20	AZT	3TC	FTC	d4T	3TC			TDF					d4T		TDF	ETR	3TC	3TC		MVC	AZT
								3TC	TDF		TDF																ABC	3TC
Rev.- Transcriptase mutations	41L	41L	41L	41L	41L	67N	41L	41L	41L	n.d.	41L	65R	67N	41L	41L	41L	41L	no	no	no	67N	41L	41L	41L	41L	67N	67N	41L
	44D	67ss	118I	44A	67N	70R	67N	67N	44D		67ss	70R	184V	44D	44D	67N	44D	data	data	data	70R	67N	44A	74V	44D	70R	70R	67N
	67N	69S	184V	67N	69D	103NS	69E	74V	67N		69S	103N	210W	67N	67N	74V	103N				103S	75I	67N	101Q	67G	215I	215I	70R
	98G	188L	215Y	75I	70R	190A	75I	101Q	101E		101Q	108I	215Y	75L	70R	98G	118I				184V	118I	75M	103N	103N	219Q	219Q	184V
	103N	215Y		103N	74V	184V	103N	184V	103N		181C	115F	219Q	118I	190A	118I	184V				190A	210W	101Q	108I	118I			
	118I			108I	103N	219Q	108I	215Y	118I		190S	151M		181C	227L	184V	210W				215F	215F	118I	181C	184V			
	210F			118I	181C		118I		184V		215Y	179E		184V	184V	210W	215Y				219Q			184V	190A	210W		
	215Y			210W	210W		178M		210W			184V		190A	210W	215Y							210W	210W	215F			
	219R			215Y	215F		210W		215Y			219E		210S	215F	227L							215F	215Y				
			219Q			215Y		219E					215Y	219R														
Protease mutations	10F	10V	10V	10I	10V	10F	10I	10I	10I	10I	10V	10I	20R	10I	10V	10F	10F	10I	10F	10I	10I	10I	10I	10F	10R	10F	10V	20I
	20I	46I	20R	33F	20R	20R	33F	53L	33F	33V	46L	20I	32I	24I	20I	33F	46I	20R	33F	20R	20R	46I	13V	46I	32I	20I	13V	36I
	36I	47V	36I	46L	33F	46I	36L	54V	46L	60E	54V	36I	46I	33F	36I	46L	54M	24I	54V	35D	36I	47V	32I	47V	33F	36I	24I	54V
	46I	71V	46I	76V	36I	54V	46L	71V	54V	76V	63P	46I	76V	46I	46I	54L	71V	36I	71V	46I	46I	71V	33F	76V	46I	46I	33F	76V
	50V	76V	76V	82F	54V	63P	76V	76V	71I		71V	54L	82A	54V	47V	71V	76V	46I	76V	54V	54V	76V	36I	84V	47V	76V	46I	82A
	54I/V	77I	90M	90M	73S	76V	82F	77I	76V		82A	76V		63P	53L	76V	82A	54V	77I	76V	71V	90M	46I	76V	84V	76V	54V	
	76V				76V	82F	84V	82A	77L		93L	84V		71V	76V	77I		76V	82A		76V		76V		84V	76V	76V	
					90M		90M	84V	82A						76V	84V	84V		82C		82F		84V	88S		82A		
							90M	90M						82A	90M	90M					90M		90M		90M			
Active Drug Scores																												
HIVdb 4.3.6	[1.5]	(2.25)	0.5	[1.75]	0.75	1.25	[1.5]	[1.75]	0.25	3.0	1.5	[0.5]	[0.75]	1.25	0.25	[0.75]	[0.5]	[0.5]	1.5	n.d.	0.5	[1.25]	[0.25]	[1.5]	0.0	[1.0]	[1.75]	[1.75]
Rega V7.1.1	2.0	(2.75)	1.5	2.0	1.0	1.0	2.0	3.5	0.0	3.0	1.75	[1.0]	[1.5]	1.0	0.0	[0.25]	[0.75]	[0.75]	1.75	n.d.	0.75	[1.0]	[0.5]	2.75	0.5	2.0	2.25	4.25
HIVGrade04/08	2.5	(3.0)	1.5	2.25	1.0	0.5	2.75	2.75	0.0	3.0	(2.0)	[1.0]	[1.0]	(2.0)	0.0	[1.0]	[1.0]	[1.0]	(2.0)	n.d.	1.0	[1.75]	[0.0]	[1.75]	0.0	[0.75]	2.0	2.0
ANRS 10/2007	3.0	(2.0)	0.5	[1.0]	1.0	1.0	2.0	2.5	0.5	3.0	1.5	[0.0]	2.0	1.0	0.5	[1.0]	[1.0]	[0.5]	(2.0)	n.d.	0.5	2.0	[0.5]	2.5	1.5	[1.5]	2.5	3.0
geno2pheno	2.0	(3.0)	1.0	2.5	(2.0)	n.d.	n.d.	2.0	n.d.	3.0	(2.0)	[0.5]	[0.5]	1.5	0.0	[1.0]	n.d.	n.d.	n.d.	n.d.	0.0	2.5	n.d.	2.0	0.0	1.0	2.0	4.0
VircoType	2.25	(2.0)	(2.0)	2.5	1.5	n.d.	n.d.	3.5	n.d.	3.0	2.5	2.0	2.0	1.0	0.5	[0.5]	[1.0]	[1.0]	n.d.	n.d.	0.5	[1.0]	[1.0]	3.0	1.5	2.0	3.0	3.5
Phenotype	2.5	n.d.	0.0	n.d.	n.d.	(3.0)	3.0	3.0*	n.d.	n.d.	n.d.	2.5	2.5	(2.0)	n.d.	n.d.	n.d.	[0.5]	n.d.	n.d.	0.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Follow-up																												
Baseline VL	1070	160000	26900	3231	47653	859000	7700	27000	35400	4248	6680	25900	4840	31400	39000	20163	8751	8800	88100	16100	310510	< 50	72100	14900	1300	744	2078	443
Lowest VL (12-96 weeks)	< 50	3410	38700	< 50	4943	190	< 50	< 50	1020	< 50	1320	< 50	< 50	116	24000	< 50	< 50	< 50	559	< 50	18270	< 50	< 50	< 50	1300	< 50	< 50	< 50
VL at week 96	13589	46570	152000	< 50	20626	LFU	LFU	LFU	16500	LFU	LFU	< 50	< 50	116	LFU	< 50	< 50	LFU	LFU	1777	LFU	391	60	< 50	LFU	< 50	< 50	< 50

Based on five genotypic resistance interpretation tools as well as two phenotypic resistance tests, an active drug score (ADS) for the follow-up therapy was calculated by adding the activity score for every active drug ranging from AS = 1.0 for complete sensitivity, AS = 0.75 for potential low-level resistance, AS = 0.5 for intermediate resistance, AS = 0.25 for possible resistance and AS = 0.0 for complete resistance. Their prediction on follow-up therapy was then compared with the virological response in a time frame of 96 weeks. Successful therapies despite an active drug score prediction of <2 are displayed in bold and square brackets. Unsuccessful therapies despite an active drug score prediction of ≥2 are displayed in round brackets. LFU=loss of follow-up.

Table 2 Comparison of results (group A and group B)

	Virus Suppression			Therapy success	
	Group A Median Viral load	Group B Median Viral load	Mann-Whitney	Group A Success	Group B Success
Baseline	26,950 (N = 10)	8,800 (N = 17)	P = 0.12	0% (N = 10)	5.9% (N = 17)
Week 12	370 (N = 10)	< 50 (N = 15)	P = 0.07	40.0% (N = 10)	66.7% (N = 15)
Week 24	4650 (N = 8)	< 50 (N = 13)	P = 0.16	37.5% (N = 8)	69.2% (N = 13)
Week 48	3410 (N = 7)	< 50 (N = 13)	P = 0.19	42.9% (N = 7)	53.8% (N = 13)
Week 96	15,045 (N = 6)	< 50 (N = 9)	P = 0.044	16.7% (N = 6)	54.5% (N = 9)

Comparison of results (group A and group B). Median viral loads and the therapy success rates illustrate a significantly better long-term suppression of HIV when SQV/ATV plus optimized backbone therapy is combined with a L76V-selecting drug. In bivariate analysis, these results were independent from slightly different baseline viral loads ($< 0.5\log$) between group A and B.

therapy failure were diagnosed positive for L90 M. The remaining two were therapy non-compliant. Thus, it might be questionable if SQV, which primarily selects L90 M should be replaced in favour of ATV [18-20]. In addition, due to the approval of new drug classes over the past years one might err on the side of caution to supplement therapy regimens with new drugs. Due to the low potency of the present cohort and varying amounts of HIV non-B infected individuals in

the examined patient groups, caution should be additionally advised, since these limitations might have effect on clinical outcomes.

Nevertheless, since there are still distinct discrepancies, mostly overestimation of resistance, in the prediction of the resistance level for Atazanavir and Saquinavir in five of the most common genotypic interpretation systems, there is still a need for further evaluation in the case of L76V occurrence.

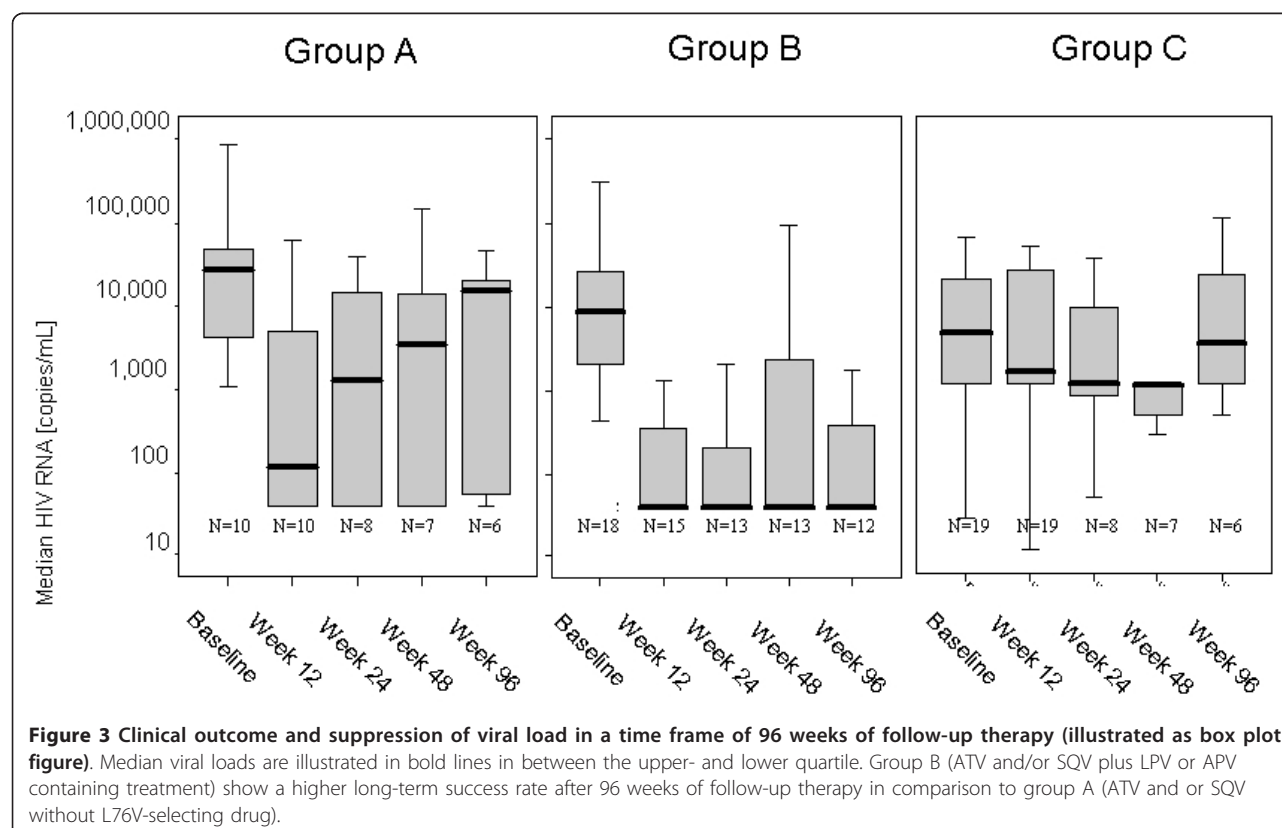


Table 3 Compensatory changes in virus genotypes within 96 weeks of follow-up therapy

Group	Patient ID	Protease mutations at start of therapy	Time of therapy failure	Time of 2nd genotype	Protease mutations after therapy failure
A	#1	L10FL, K20I, M36I, M46I, I50V, I54IV, L63P, L76V	Week 48	Week 144	L10F, <u>V11I</u> , <u>I13V</u> , K20R, <u>V32I</u> , <u>L33F</u> , M36I, M46I, <u>I47V</u> , <u>I54M</u> , <u>L63P</u> , <u>A71V</u> , <u>G73S</u> , <u>I84V</u> , <u>L90M</u>
	#2	L10V, M46I, I47V, L63P, A71V, L76V , V77I	Week 12	Week 48	L63P, V77I (therapy interruption)
	#4	L10V, K20RK, M36I, M46I, L63P, L76V, L90M	Week 12	Week 24	L10V, K20R, M36I, M46I, <u>F53L</u> , L63P, <u>I84IV</u> , L90M
	#22	L10I, L33F, M46L, I54V, L63P, A71I, L76V , V77I, V82A, L90M	Week 12	Week 48	L10I, L33F, M46L, <u>F53L</u> , I54V, L63P, A71T, <u>G73S</u> , V77I, V82A, L90M
B	#8	K20R, V32I, M46I, L76V , V82A	Week 48	Week 48	K20R, V32I, <u>M36I</u> , M46I, <u>F53FL</u> , L76V , V82A, <u>L90LM</u>
	#9	L10I, L24I, L33F, M46I, I54V, L63P, A71V, L76V , V82A	Week 12	Week 24	L10I, L24I, L33F, M46I, <u>F53L</u> , I54V, L63P, A71V, L76V , V82A, <u>I84V</u>
	#10	L10IV, K20I, M36I, M46I, I47V, F53L, L63P, A71V, G73 D, L76V , I84V, L90M	Week 12 compliance	Week 48	L10V, K20I, <u>L33I</u> , M36I, M46I, I47V, F53L, L63P, A71V, G73 D, L76V , I84V, L90M
	#27	L10I, M46I, I47V, L63P, A71V, L76V , L90M	Week 48	Week 48	L10I, M46I, I47V, L63P, A71V, L76V , <u>I84V</u> , L90M
C	#6	L10V, L33F, M46L, I54V, A71V, L63P, A71V, L76V , V82A	Week 12	Week 96	L10V, <u>K20R</u> , L33F, <u>M36I</u> , M46L, I54V, A71V, L76V , V82A
	#23	L10FIRV, L33F, I54MV, D60E, L63P, A71V, L76V , V82F	Week 12	Week 24	L10FIRV, L33F, I54MV, D60E, L63P, A71T/V, L76V , V82F

Compensatory changes in virus genotypes within 96 weeks of follow-up therapy. Patients with failing therapies within the 96 weeks received a second resistance testing. While L76V was still present in patients receiving L76V-selecting drugs, it was then absent in patients without these drugs (new detected mutation are underlined). Therapy failure in group B was noticeable associated with an additional establishment of the protease mutation L90 M.

Methods

Clinical material

HIV strains of 46 intensely pretreated (34 showed NRTI/NNRTI/PI resistance/12 showed NRTI/PI resistance) and one naïve with transmitted mutation, L76V-positive patients derived from 24 centres throughout Germany between 1999-2009 were retrospectively analysed for HIV-resistance patterns and success of follow-up therapy. The inclusion criterion is provided in Figure 4. Descriptive statistics concerning person-to-person variations of virological and immunological parameters were assessed at baseline before switch of therapy and are provided in Table 4.

All patient data was categorized in three groups concerning the follow-up therapy:

Group A: ATV and/or SQV (no selection pressure on L76V)

Group B: LPV or APV plus ATV or SQV (maintained selection pressure on L76V)

Group C: LPV or APV plus other drugs (maintained selection pressure on L76V)

All patients received an optimized backbone therapy.

HIV-1 RNA Quantification

Plasma of patients was analysed at baseline and week 12, 24, 48 until end of investigation at week 96 to monitor efficiency of therapy. Plasma RNA was measured by using the COBAS AMPLICOR HIV-1 Monitor system and the Abbott m2000sp/rt system according to the manufacturer's recommendations.

Genotypic resistance testing

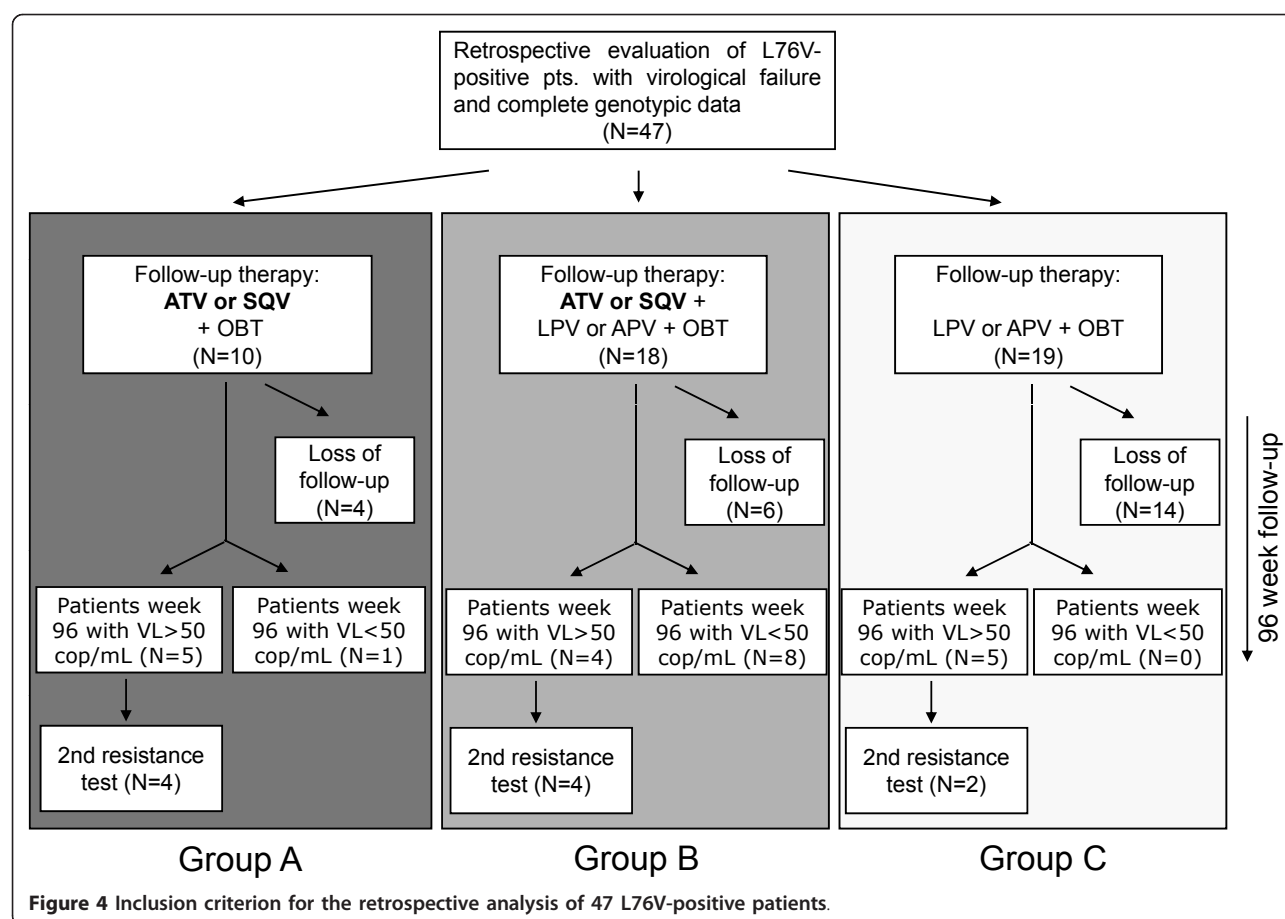
Plasma samples of all 47 patients were collected and stored at -20°C until time of RNA extraction. All specimens were processed by using the FDA-approved Siemens HIV TruGene system as well as the Abbott HIV-1 Genotyping System on the Applied Biosystems' 3100 capillary electrophoresis platform according to the manufacturer's recommendations. HIV-1 genotypes were processed and analyzed by using the wildtype LAV-1 sequence as reference. The sensitivity for detecting minor quasispecies variants was 15%. In this cohort, only patients with major L76V positive population were included. Minor wildtype variants were not detected at this position.

Phenotypic resistance testing

Phenotypic resistance analysis of the complete protease gene and the first 900bp of the RT were performed according to an earlier described recombinant virus assay by determining a virus specific resistance factor [21]. In addition, the Virtual Phenotype™ (based on 53,000 paired genotypes and phenotypes) from Virco was assessed for those patient samples where no recombinant virus assay was realizable.

Interpretation of drug resistance

Several algorithms are available worldwide, both in public and private domains. The concordance of resistance predictions was analysed between the five most commonly used algorithms [REGA v7.1.1 [22] HIVGrade



ver.12/2008 [23] ANRS ver.10/2007 [24] Stanford HIVdb ver.4.3.6 [25] and the geno2pheno online tool [26]] for the drugs ATV, SQV, LPV and/or APV. Multiple resistance tests in treatment history were cumulatively documented. In addition, an active drug score (ADS) was

determined in order to analyze the amount of remaining active drugs in follow-up therapies of each patient (susceptible = +1/low intermediate = +0.75/intermediate = +0.5/high intermediate = +0.25/resistant = +0.0). This ADS allowed a statement concerning the prediction of

Table 4 Patient characteristics and parameters

Parameter	Total	Group A (N = 10)	Group B (N = 18)	Group C (N = 19)
Gender				
Male	84%	100%	100%	68,7%
Female	16%	0%	0%	31,3%
HIV-1 subtype				
Patients with subtype B	69%	80%	64%	67%
Patients with non-B subtype	31%	20%	36%	33%
Treatment history				
Mean duration under ART in months (mean)	66	80	88	54
Current active drug score (mean)	-	1.5	1.3	0.5
HIV-1 RNA [copies/ml; median]				
Baseline	20,163	26,950	8,800	42,600
CD4 cell counts [cells/μl; median]				
Baseline	260	291	307	246

Patient characteristics and parameters. All patients were categorized in three groups as described throughout the article: Group A (ATV and or SQV), group B (ATV and or SQV plus L76V selecting drug LPV, APV or DRV) and group C (L76V selecting drug plus optimized backbone therapy).

follow-up therapy. It is generally accepted that a successful therapy should contain at least two active drugs, preferably three (ADS ≥ 2.0) [27,28].

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Authors' contributions

FW has made substantive intellectual contribution to the study including acquisition-, analysis- and interpretation of data and finally drafting the manuscript. JV assisted as consultant in patient-specific aspects and was involved in manuscript revision. GN was responsible with genotyping processes as described in the manuscript. RE was responsible for genotypic resistance interpretation and manuscript revision. HW realized the phenotypic resistance analysis. PB and AT assisted in concept and design aspects and directed sample- and data acquisition. HK, RK, MS and TB were significantly involved in data acquisition, provision of samples and manuscript revision. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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